

## REMARKS

### **I. Examiner Interview**

Applicants thank the Examiner and his mentor, Rebecca Prouty, for their time during the May 20, 2008, Examiner Interview. Applicants' counsel, Paul Prendergast and Cara Crowley-Weber, initiated the Interview to discuss the April 4, 2008, Office action. In particular, Applicants' counsel and the Examiner agreed the § 102(b) rejection should have been a § 102(e) rejection. Also discussed was the possibility of amending the independent claims to reflect the organism the hydrogenase is derived from to overcome § 112, second paragraph, issues.

### **II. Claim Amendments**

Claims 1, 30-42, and 45 are pending in the case. Claims 1, 30-35, and 40 are amended. Specifically:

Claims 1, 35, and 40 are amended to indicate the oxygen-resistant iron hydrogenase is derived from *Chlamydomonas reinhardtii*. This amendment can be supported by Applicants' specification at, for example, paragraphs [0036] and [0040]. Claims 30-34 are amended to reflect proper claim dependency. This amendment corrects an obvious typographical error. Applicants believe that no new material has been added.

All references to the specification in this amendment refer to Application Publication No. 2006/0228774. Applicants respectfully request reconsideration of the application as follows:

### **III. Claim Rejections under 35 U.S.C. § 112, First Paragraph**

Claims 1, 30-42, and 45 are rejected for allegedly failing to comply with the enablement and written description requirements. While Applicants respectfully disagree, in order to expedite prosecution the claims are amended to address the Examiner's concerns.

### **IV. Claim Rejections under 35 U.S.C. § 102(e)**

Per the Examiner Interview and subsequent Interview Summary, the § 102(b) rejection was changed to a § 102(e) rejection. As such, the Examiner asserts claims 1, 31-42, and 45 are anticipated by Dillon *et al.* (U.S. 2007/0009942) under 35 U.S.C. § 102(e). Applicants respectfully disagree.

Claim 1 as amended is directed to an oxygen resistant iron hydrogenase derived from *Chlamydomonas reinhardtii* by substitution of one or more identified amino acid residues within

a hydrogen channel of the oxygen-sensitive iron hydrogenase. The one or more identified amino acid residues are independently substituted with a naturally occurring or synthetic amino acid selected from the group consisting of tryptophan, isoleucine, leucine, phenylalanine, and derivatives thereof. The resulting oxygen-resistant hydrogenase limits O<sub>2</sub> diffusion through the channel while allowing H<sub>2</sub> diffusion out of the channel, and, relative to the oxygen-sensitive hydrogenase, has a reduced channel diameter.

Dillon is allegedly directed to a method for evolving an iron hydrogenase by substituting any amino acid within the sequence  $\text{FX}^1\text{X}^2\text{X}^3\text{G}^1\text{G}^2\text{VMEA}^1\text{A}^2\text{X}^4\text{R}$  of an iron hydrogenase with any amino acid, transforming an organism with a nucleic acid encoding the modified iron hydrogenase, then screening the organism for the ability to produce hydrogen in the presence of oxygen.

Dillon fails to anticipate Applicants' claim 1. Chisum on Patents, § 3.02[b], indicates the law is well settled on the matter that a valid patent may issue for a non-obvious improvement on a prior invention, even though the improvement falls within the claims of that prior patent. Chisum further indicates that, as a result, a prior genus which does not explicitly disclose a species does not anticipate a later claim to that species. *Id.* See also, *Minnesota Mining & Co. v. Johnson & Johnson*, 976 F.2d 1559, 1572 (Fed. Cir. 1992). Dillon proposes that any one of the 13 amino acids in the above-mentioned sequence can be substituted with any amino acid. This genus is large: substitution with any amino acid, even if each evolved hydrogenase has only one substitution at a time, generates a minimum of 260 evolved hydrogenases. Claim 1, on the other hand, describes substitution with specific, functionally relevant amino acids, for example, tryptophan, isoleucine, leucine, or phenylalanine. Thus, at least because Dillon does not explicitly disclose the species encompassed by claim 1, Dillon does not anticipate claim 1.

Claims 31-34 depend from claim 1 and are therefore novel for at least the same reasons as described above with respect to claim 1.

Claim 35 is directed to an oxygen-resistant iron hydrogenase with a hydrogen channel diameter defined by one or more diameter defining amino acid residues. The diffusion of oxygen within the channel is reduced relative to oxygen diffusion in the hydrogen channel of an oxygen-sensitive iron hydrogenase. The diameter defining residues are selected from the group of tryptophan, isoleucine, leucine, phenylalanine, and their derivatives.

Dillon fails to anticipate claim 35. As described above, Dillon mentions that any one of the 13 amino acids in the sequence  $\text{FX}^1\text{X}^2\text{X}^3\text{G}^1\text{G}^2\text{VMEA}^1\text{A}^2\text{X}^4\text{R}$  can be substituted with any

amino acid. As described above, substitution with any amino acid, even if each evolved hydrogenase has only one substitution at a time, generates a minimum of 260 evolved hydrogenases. Claim 35, on the other hand, describes substitution with specific, functionally relevant amino acids, for example, tryptophan, isoleucine, leucine, or phenylalanine. Thus, at least because Dillon does not explicitly disclose the species encompassed by claim 35, Dillon does not anticipate claim 35.

Claims 36-39 depend from claim 35 and are therefore novel for at least the same reasons as discussed above with respect to claim 35.

Claim 40 is directed to an oxygen resistant iron hydrogenase having one or more identified amino acid residues within the hydrogen channel substituted to reduce the oxygen sensitivity. The one or more identified amino acid residues are independently substituted with an amino acid selected from the group consisting of tryptophan, isoleucine, leucine, phenylalanine, and their derivatives, and the substituted amino acid has properties that limit O<sub>2</sub> diffusion through the channel while allowing H<sub>2</sub> diffusion out of the channel. The substituted amino acid also reduces the diameter of the channel.

Dillon fails to anticipate claim 40. As described above, Dillon mentions that any one of the 13 amino acids in the sequence FX<sup>1</sup>X<sup>2</sup>X<sup>3</sup>G<sup>1</sup>G<sup>2</sup>VMEA<sup>1</sup>A<sup>2</sup>X<sup>4</sup>R can be substituted with any amino acid. As described above, substitution with any amino acid, even if each evolved hydrogenase has only one substitution at a time, generates a minimum of 260 evolved hydrogenases. Claim 40, on the other hand, describes substitution with specific, functionally relevant amino acids, for example, tryptophan, isoleucine, leucine, or phenylalanine. Thus, at least because Dillon does not explicitly disclose the species encompassed by claim 40, Dillon does not anticipate claim 40.

Claims 41, 42, and 45 depend from claim 40 and are therefore novel for at least the same reasons as discussed above with respect to claim 40.

#### **V. Considerations under 35 U.S.C. § 103**

Further, Applicants' claims are not obvious over Dillon. Applicants carefully analyzed the HydA1 H<sub>2</sub>-channel sequence and structure, replacing one or more identified amino acid residues lining the interior of the channel with specific amino acids such as tryptophan, isoleucine, leucine, or phenylalanine. See Examples 1 and 2. Microorganisms such as *C. reinhardtii* transformed as described in Example 2 and tested for hydrogenase activity in the

presence of oxygen uniquely exhibit higher fold enzymatic activity relative to wild-type cells under similar conditions. See Example 3. Moreover, testing their findings *in silico* demonstrated, surprisingly, that individual mutations and combined mutations reduced the average overall channel diameter. See Example 1 and Tables 1 and 2. Dillon neither teaches nor suggests substitution with the amino acids tryptophan, isoleucine, leucine, or phenylalanine, thus, for at least these reasons, claims 1, 30-42, and 45 are non-obvious over Dillon.

**VI. Preservation of Right to Remove Reference**

Although Applicant believes that the claims are sufficiently differentiated from the Dillon reference, Applicant hereby reserves the right to swear behind the Dillon reference if these or similar rejections based on the Dillon reference is maintained in a subsequent Office Action.

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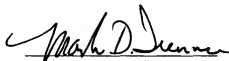
For the reasons set forth above, Applicants respectfully submit the claims are allowable and reconsideration and issuance of a notice of allowance are respectfully requested. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 14-0460 if not otherwise specifically requested. The undersigned hereby authorizes the charge of any required fees not included or any deficiency of fees submitted herewith to be charged to deposit account No. 14-0460.

Respectfully submitted,

Date:

7-2-2008



Mark D. Trenner, #43,961  
National Renewable Energy Laboratory  
1617 Cole Blvd.  
Golden, CO 80401  
(303) 384-7499